American Journal of Microbiology and Biotechnology
2015; 2(3): 38-43
Published online May 20, 2015 (http://www.aascit.org/journal/ajmb)
ISSN: 2375-3005

Keywords
Candida Spp,
Catheters,
Fungal Infectivity,
Biofilms,
SEM

Received: April 3, 2015
Revised: April 18, 2015
Accepted: April 19, 2015

Nosocomial Fungal Infectivities: *In Vivo* Formation of *Candida* Biofilms on Catheters Surfaces

Sidi Mohammed Lahbib Seddiki[1, 2, *], Zahia Boucherit-Otmani[1], Reda Ali Bettioui[3], Kebir Boucherit[2], Mourad Taleb[4], Dennis Kunkel[5]

[1]Laboratory Antifungal Antibiotic, Physico-chemical synthesis and biological activity, University of Tlemcen, Imama, Algeria
[2]University Center of Naâma, Institute of Sciences, Dept. of nature and life sciences, Naâma, Algeria
[3]Laboratory: Actions of the human for the protection of the environment and application in public health, department of ecology and environment, University of Tlemcen, Imama, Algeria
[4]Department of Epidemiology and preventive medicine, University Hospital of Sidi Bel Abbes, Algeria
[5]Dennis Kunkel Microscopy Inc, Kailua, USA

Email address
seddiki.med@gmail.com (S. M. L. Seddiki)

Citation
Sidi Mohammed Lahbib Seddiki, Zahia Boucherit-Otmani, Reda Ali Bettioui, Kebir Boucherit, Mourad Taleb, Dennis Kunkel. Nosocomial Fungal Infectivities: *In Vivo* Formation of Candida Biofilms on Catheters Surfaces. *American Journal of Microbiology and Biotechnology*. Vol. 2, No. 3, 2015, pp. 38-43.

Abstract
The invasive nosocomial infections due to *Candida* species are responsible for increasing the length of stay, cost of hospitalization and morbidity in immunocompromised patients. Their severity and rapid progressivity are owed to the difficulty of diagnosis. Various catheters, which are often used to train a body fluid (blood, urine, infusion, parenteral nutrition, medication ...) inside the body of the patient or vice versa, are susceptible to be altered by *Candida spp.* and promote the formation of biofilms which consolidates the risk of invasive nosocomial infections; i.e., these structures are considered as a nest for disease because it is not easily eradicate by conventional antifungal therapy. Such as the diagnosis of candidiasis related to catheter is difficult, the differentiation between catheter infection and a simple contamination is essential to establish an antifungal treatment. This study aimed adapts to yeasts the Brun-Buisson (1987) method which only concerned by bacteria, it's why we conducted our study between February 2011 and January 2012 at the Hospital University Center of Sidi Bel Abbès-Algeria that aims to evaluate the various types of fungal catheters infectivities (contaminations, colonization and infections) and their corresponding rates, as well as the responsible yeast species. At the end, the ability to form biofilms was checked. The results showed that three types of fungal infectivities of catheters were identified. On the other hand, SEM images showed clearly *Candida* biofilms on the surfaces of catheters.

1. Introduction

Nosocomial invasive fungal infections, including those caused by *Candida* species, are a major cause of morbidity and mortality in immunocompromised patients[1,2]. These infections are usually severe, rapidly scalable and difficult to diagnosis and to treat[3].

In conjunction with the medical advances, the extensive use of different types of

catheters in hospitals has grown significantly in recent decades[4]. However, nosocomial infections due to *Candida* spp. increase in parallel with the use of these devices[5,6].

Indeed, more than half of these infections are related to the presence of catheters[7], which are responsible for an increase in the length of stay, cost of hospitalization and morbidity[8].

On the other hand, the emergence of non-albicans species of *Candida* in hospital, like *C. glabrata, C. parapsilosis* and *C. tropicalis*, is regularly observed in the last two decades[9,10]. Without a doubt, epidemiological changes are linked to the frequency of fungal nosocomial infections, the involved species and the development of resistance to conventional antifungal agents, i.e., amphotericin B and triazole[11]. Furthermore, *Candida* spp. may colonize the catheter tip and form biofilms[12-14], which is a real risk to patients[7]. So, the presence of the catheter is a major risk factor for the development and persistence of candidal nosocomial infection[15].

In addition, the differentiation between the catheter infection and its contamination is essential to establish an antimicrobial treatment[16,17]. According to[16,18], it is important to differentiate between colonization, contamination and infection of catheters by culturing its distal end.

Usually, two methods are adopted for the evaluation of catheter-related infections, the semi-quantitative method[19] and the quantitative method[20]. However, these methods process only catheters altered by bacteria.

Seddiki et *al* (2013) distinguish three types of alterations catheters caused by *Candida* spp. They termed these alterations "types of catheter infectivity" which refer to the degree of infectiveness of catheters.

The objective of this study is to isolate yeasts from various catheters after their ablations from admitted patients to different hospital units in Sidi Bel Abbes (Algeria), to respond to the request of biologists concerning the appropriate method to make the difference between infection, colonization and contamination of catheters and to evaluate the ability of isolated strains to form biofilms on their surfaces.

2. Materials and Methods

2.1. Sampling and Identification

Samples were taken between February 2011 and January 2012 from ten services at the University Hospital of Sidi Bel Abbes (ICU, pediatric surgery, general surgery, neurosurgery, traumatology, urology, gastroenterology, pneumophtisiology, clinical hematology and endocrinology). The concerned patients are those with significant risk factors for candidiasis (implanted medical devices, broad spectrum of antibiotic therapy, long stays in service, invasive procedures ...). For each patient, data were collected using a datasheet (Table 1).

Table 1. *Technical data sheet for the information collection during each sampling.*

Hospital:	**Service:**	
Name		
First Name		
Gender	Male ❑	Female ❑
Age		
Adress		
Phone Number		
Number of patients in the same room :		
Number of the beds :		
Admission date in hospital :		
Date of catheter insertion :		
Sampling date :		
Date of discharge from hospital :		
Origin of the sample	Catheter ❑	Type :
	O ther ❑	Specify :
Immunosuppression	Yes ❑	Specify:
	No ❑	
Antibiotic therapy	Yes ❑	Specify:
	No ❑	
Evolution of disease	Healing ❑	
	Death ❑	
	Unknown ❑	
Isolated strains:		

According to[22,23], only implanted catheters for 48 hours or more were taken. All types of catheters were include in this study, sampling was carried out from different catheters (PVC: Peripheral Venous Catheter, CVC: Central Venous Catheter, UC: Urinary Catheter, TC: Tracheotomic Catheter, OC: Orobronchique Catheter and DC: Drainage Catheter). By taking measures of asepsis, each catheter was removed separately, its distal end was cut (3-5 cm long) and then placed in a sterile tube containing 1 mL of physiological saline. Purification of yeast was performed by successive subcultures on Sabouraud medium.

Identification of purified strains was based on their morphological and biochemical characteristics. Microculture testing (germ-tube and chlamydospore formation) and the yeast identification system (Api Candida® System; bioMériux, Marcy L'Etoile, France) were used.

2.2. Types of Fungal Catheters Infectivities

Since 2003, the department of epidemiology and preventive medicine of the University Hospital of Sidi Bel Abbes has adopted a strategy for the surveillance of nosocomial infections by repeated prevalence surveys in order to measure their rates and track their evolutionary trends. The prevalence survey conducted in 2009 showed that 56.8% of patients with nosocomial infection had a peripheral venous catheter, 11.4% had central venous catheter and 13.6% with other types of catheters. In addition, broad-spectrum antibiotics were often used in different services, while antifungals were rarely prescribed (unpublished data).

The distinction between infection, colonization and contamination of catheters was assessed by referring to the significance threshold (10^3 cells/mL) and the collected data taken during the sampling (systemic and/or local clinical symptoms)[17,20,24].

The segments of catheters which exhibit a negative result were transferred to new tubes containing liquid of Sabouraud medium supplemented of 50 mg/L of chloramphenicol and then incubated at 30 °C for 24 to 48 hours or even to 72 hours.

The results of different types of fungal infectivities were made subject to a statistical study, the values of variances (*P*) less than 0.05 were considered significant.

2.3. SEM Observation of Biofilms

To demonstrate the ability of isolates to form biofilms and to observe their structures, three segments of different catheters were taken to examine them in scanning electron microscope (SEM).

The choice of segments was performed following the light microscopic observations of suspensions in which the segments catheters were submerged. Observation of pseudo-mycelial and mycelial structures or substantial clumps of cells allowed predicting the existence of biofilms on the surfaces of the catheters.

To carry SEM photographs, samples were fixed with glutaraldehyde and then sent to the laboratory Dennis Kunkel Microscopy. Inc., USA. The samples were examined using a Hitachi S-4800 (Hitachi Ltd, Tokyo, Japan) field-emission SEM.

The fixation consisted of cutting segments catheters of 7 to 8 mm long in Eppendorf tubes containing 1 mL of PBS buffer saline, 10 mM (2.7mM KCl, 137 mM NaCl, pH 7.4) and glutaraldehyde at a final concentration of 2.5%. The tubes were placed in a shock chamber and left at 4 °C before shipment.

3. Results and Discussion

3.1. Types of Fungal Catheters Infectivities

Among 457 taken samples, 37 strains of *Candida* spp. were isolated, i.e., a rate of 7.79%. Contaminations of catheters were dominant with a rate of 55.56%. They were related essentially to *Candida glabrata* at a rate of 33.33%. The second concerned species in this type of infectivity was *C. albicans* (16.66%) whereas *C. parapsilosis* was responsible only for 5.55% of contaminations. Colonizations of catheters were less important than contaminations (30.56%). *C. glabrata* was responsible for 22.23% of colonized catheters; however, 5.56% were caused by *C. albicans* and only 2.78% by *C. parapsilosis*. On the other hand, the rate of catheters infections was 13.88% where *C. glabrata* and *C. albicans* were responsible respectively for 8.33% and 5.56% of this type of infectivity (Figure 1).

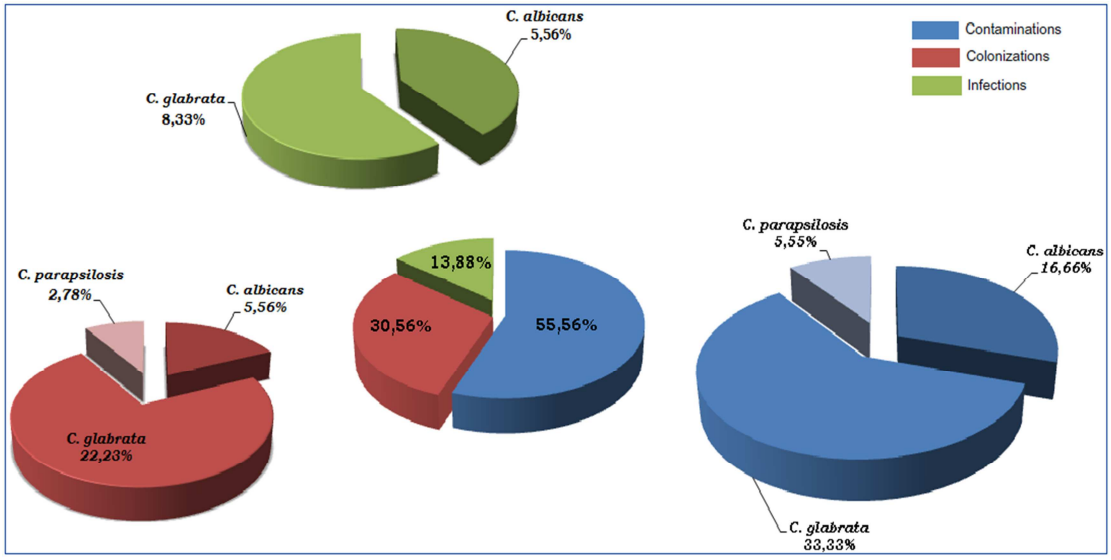

Figure 1. *Types and rates of fungal catheters infectivities at the university hospital of Sidi Bel Abbes - Algeria.*

In this study, the ICU ranks first with regard to infections of catheters with a rate of 4.76% followed by the pneumophtisiology service (2.38%) and then, the general surgery department which was ranked third (2.13%). Yet, this service was relatively the most responsible for catheters colonizations with a rate of 8.51%, followed by the pediatric surgery service with 4.44%. However, the ICU ranks third for this type of infectivity (3.18%), although, it ranks first with 9.52% of contaminations catheters. In addition, it was found that two to three types of fungal infectivities were observed in different services, except for the pediatric surgery and clinical hematology services where only the catheters colonizations were distinguished. Statistical analysis reveals a significant difference between the studied services (P = 0.01), and a clear difference between the types of fungal infectivities (P = 0.03).

The World Health Organization[25] estimated that 5% to 12% of patients in the world develop a nosocomial infection, while more than 60% are associated with the implantation of a medical or surgical device especially the catheters. Indeed, nosocomial infections can occur at any time during the use of catheters[24]. Thus, our results are consistent with others[14,26-29] which showed that the use of catheters is the main source of invasive infections in hospitalized patients,

particularly in ICU. In addition, the frequency of fungal microorganisms causing nosocomial infections varies from one country to another according to the institutions, the intensive care, the antibiotic protocols and the type of catheters used[30-32].

In fact, our results demonstrated that the types of fungal infectivities vary depending on the type of catheters used. The Central catheters were the most implicated in infections with a rate of 18.18%; this could be explained by several factors including the duration of implantation greater than 8 days and up to 21 days[16,33]. However, the drainage and orobronchial catheters were responsible respectively for 5.88% and 5.4% of colonizations. For infections, the orobronchial catheters were essentially responsible (2.7%). Additionally, a significant difference between the types of catheters used was noted (P = 0.04). This result is not in agreement with those of[34,35] that showed catheters infections were related to the presence of CVC.

3.2. SEM Observation of Biofilms

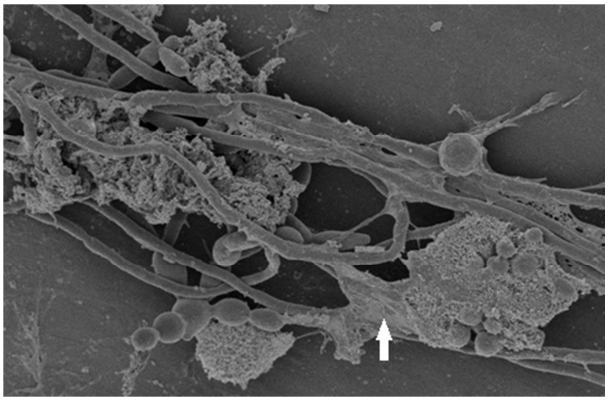

Figure 2. *SEM morphology of Candida albicans "HFKT1" biofilm developed on the inner surface of a peripheral vascular catheter. The arrow indicates the extracellular matrix (Magnification × 2000).*

Figure 2 shows a biofilm formed by the isolated strain *C. albicans* "HFKT1" from a peripheral venous catheter from the clinical hematology department after an implanted time of 5 days. The extensions of the hyphae of the biofilm are stretched on the inside of the catheter; the yeast cells and the extracellular matrix are clearly identifiable (Figure 2).

For *C. glabrata* "RFKT1" isolated from ICU, the biofilm structure is clearly visible. It is grown on the inner face of a vascular catheter device after an implanted time of two days, it is formed of blastospores were encapsulated in extracellular matrix (Figure 3.)

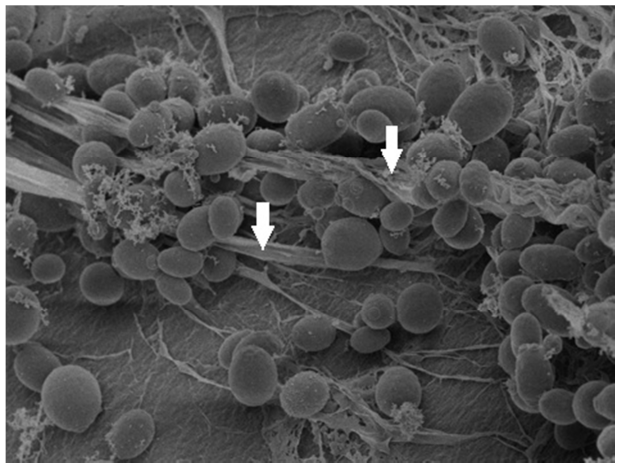

Figure 3. *SEM morphology of Candida glabrata "RFKT1" biofilm developed on the inner surface of a peripheral vascular catheter. Arrows indicate the extracellular matrix (Magnifications × 4500).*

Figure 4 represents a peripheral venous catheter removed from the general surgery service after an implanted time of three days. The external surface of this catheter (A) is not contaminated and there is no yeast form, while the inner surface (B) contains blastospores which initiate the formation of a biofilm (the initial adhesion stages of biofilm formation of *C. albicans* "CFKT3"). Extracellular matrix surrounds and develops the young biofilm (Figure 4).

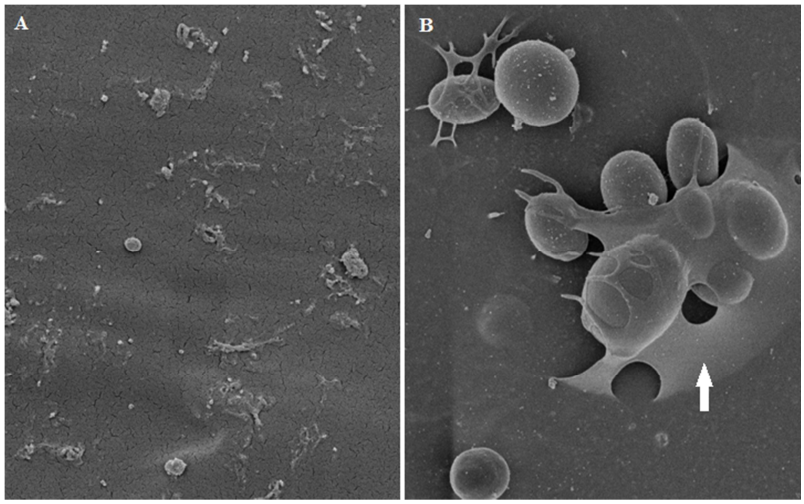

Figure 4. *SEM of (A) the outer surface of catheter not colonized (B) the early stage of biofilm formation of Candida albicans "CFKT3" on the inner surface of the same catheter. Arrows indicate the extracellular matrix (Magnifications: A ×15 000, B ×6000).*

It is well established that many *Candida* species produce biofilms, most fungal clinical manifestations that are associated with the use of catheters are associated with biofilms formation[36,37]. The diagnosis of the infected catheter and the research of implicated yeasts are improved by performing a review SEM[38]. This review, indeed, provides detailed topography of biofilms at high magnification image[39]. In addition, the three-dimensional structure of the biofilm is important for understanding the physiology and ecology of their microbial system[40]. Biofilms of *Candida* spp. are considered as a functional consortium in which fungal cells enveloped by an extracellular matrix[41,42].

On the other side, mature biofilms of *Candida albicans* consist of yeast, hyphae and pseudohyphae[43,44]. In contrast, the biofilm of *C. glabrata* is monomorphic; it is formed only of blastospores enclosed in an extracellular matrix[45].

4. Conclusion

Three types of fungal infectivities of catheters were identified in Sidi Bel Abbes University Hospital. Contamination ranks first with a rate of 55.56%, followed by colonization (30.56%) and then by infections together with a rate of 13, 88%.

475 samples were taken, 37 strains (7.79%) of *Candida* spp. were isolated, along with the dominance of *Candida glabrata*. The distribution of strains varied from one service to another. The ICU ranks first with a rate of 19.05% followed by general surgery (14.89%). The neurosurgery and clinical hematology rank last with 2.27% and 2.32% respectively. The adapted Brun-buisson (1987) method to yeasts seems to be appropriate to assess the types of fungal catheter infectivity. It offers to clinicians the opportunity to make a treatment decision in a very short time, especially if there is infection of the catheter. The use of Thoma cells for counting yeasts, without waiting the microbial culture which needs a minimum period of 24 hours, allows save time. On the other hand, 31/37 isolated strains had the potential to form biofilms, those structures presented mainly significant candidiasis risk factors. The images of the SEM show the biofilms formed by *Candida* spp. on the internal surfaces of the catheters. The three-dimensional biofilm structure of this yeast was formed of aggregate of blastospores, pseudohyphae and hyphae encapsuled in an extracellular matrix.

To conclude, it is highly recommended to be so vigilant when using catheters to prevent fungal infections; the essential recommendation is the limitation of the indications and the duration of catheterization. Thus, manipulations on intravenous lines should be minimized.

Acknowledgments

The authors would like to thank Abdelali AGOUNI, Lecturer in Cardiovascular Biology at University of Surrey, Faculty of Health and Medical Sciences, England, UK, for his help and support.

The thanks of the authors are also addressed to Asma KEBIRI and Sabeur BENKRALED, teachers of English at the University center of Naâma, for reviewing the article language.

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